# TOS PROTOCOL AND PROCEDURE: TCK – TICK AND TICK-BORNE PATHOGEN SAMPLING

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See configuration management system for approval history.

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# Change Record

| REVISION | DATE | ECO # | DESCRIPTION OF CHANGE |
|---|---|---|---|
| - | 05/16/2011 | ECO-00151 | Draft protocol |
| A | 10/03/2011 | ECO-00280 | Updated draft after 2011 field season |
| B | 07/12/2012 | ECO-00497 | Updated draft for 2012 field season |
| C | 01/10/2014 | ECO-01139 | Updated draft for 2013 field season |
| D | 03/19/2014 | ECO-01669 | Production release, template change, and other changes as detailed in Appendix C (only in rev D) |
| E | 10/01/2014 | ECO-02321 | • Migration to new protocol template<br>• Contingent decisions updated to inform responses to site and plot level delays that are acute (FOPS-1228, FOPS-1582, FOPS-1629, FOPS-1241, FOPS-1171, FOPS-1018) and delays that may be more chronic and require consideration of dropping/replacing one or more sampling plots (FOPS-1568, FOPS-1365, FOPS-1224)<br>• SOP A: format for internal sample vial labels changed (locality label format no longer used)<br>• SOP B: Text added to clarify what was formerly the “>50% draggable” rule and more clearly define when to use dragging versus flagging (FOPS-1188, FOPS-1183, and FOPS-1170). This text also provides more explanation of the efficacy of dragging vs. flagging in tall grass or where understory vegetation prevents the cloth from touching the ground (in response to FOPS-1167, FOPS-838). The text further explains how to modify sampling when “difficult veg” (including water) is encountered along the sampling path (addresses FOPS-1227). The total distance that can be sampled as each plot has been modified accordingly, and new figures are included here. Ticks of all three life stages can now be stored and shipped in the same sample vial(s) (versus previously, larvae were separate from adults/nymphs). Text has been added to clarify how frequently larvae should be rinsed from the reusable lint rollers during sampling in a plot. Text was added in response to FOPS-1566 (when to use |

| REVISION | DATE | ECO # | DESCRIPTION OF CHANGE |
|---|---|---|---|
| | | | masking tape vs. sticky buddy methods to collect larval ticks) indicating that reusable lint rollers should be used to collect larval ticks unless NEON HQ science staff have approved use of masking tape method. VialID format has been modified slightly to create unique identifiers for each sample vial. In response to FOPS-1478, siteID has been added to the datasheet.<br>• SOP C: Text was added in response to FOPS-1566 (when to use masking tape vs. sticky buddy methods to collect larval ticks) indicating that reusable lint rollers should be used to collect larval ticks unless NEON HQ science staff have approved use of masking tape method. VialID format has been modified slightly to create unique identifiers for each sample (here, ticks on masking tape attached to cardboard cards)<br>• SOP D: Ticks of all three life stages can now be stored and shipped in the same sample vial(s) (versus previously, larvae were separate from adults/nymphs) (addressed FOPS-1574). Information on and format of lab on internal vial label has been changed (was locality label, now vialID)<br>• SOP E: format of shipping manifest has been adjusted with the addition of fields and changes to the name and format of some existing fields |
| F | 03/17/2015 | ECO-02564 | Update of tick TOS protocol based on 2014 field experience and budget analysis. Details of the changes are located in the change record. |
| G | 01/29/2016 | ECO-02905 | Effective starting 2016 field season:<br>• Larval collection uses tape only (NEON-247).<br>• Changed storage<br>• preservative from 95% ethanol to RNA stabilization solution (NEON-350).<br>• Ticks found outside the plot may be discarded instead of released.<br>• Low-intensity sampling frequency resumes after a year of no ticks (NEON-354).<br>• Added instructions to maintain a narrow sampling path (NEON-554).<br>• Removed bout from sampleID format.<br>• Internal labels should be printed on all-weather copy paper and inserted inside vials. |

| REVISION | DATE | ECO # | DESCRIPTION OF CHANGE |
|---|---|---|---|
| | | | • Reduced sizes of collection tubes to 1.5-2 mL (existing supplies may be used until depleted).<br>• Distilled redundant information, restructured for clarity. |
| H | 02/17/2017 | ECO-04421 | Effective starting 2017 field season:<br>• MODIS-based sampling windows, edited site-specific sampling schedules<br>• Added alternate plot establishment protocol (NEON-1514)<br>• Changed suggested tube numbers (NEON-1744)<br>• Changed shipping materials to wet ice instead of dry (NEON-2768)<br>• Specified inclusion of 'blank' preservative in shipment (NEON-1617)<br>• Clarified instructions in case of delay when plot is partially sampled (NEON-1848)<br>• Updated template.<br>• Replaced vialID with sampleID and number of vials.<br>• Added language to accommodate mobile data applications.<br>• Added plot reallocation section and moved instructions from SOP a into this section.<br>• Reformatted SOPs for enhanced readability.<br>• Added new guidelines for shipping inventories. |
| J | 02/20/2018 | ECO-05256 | Adding barcode specific language to the protocol; adjusting date range to a window (NEON-5942) |
| K | 03/18/2019 | ECO-05962 | • Update Appendix D based on most recent available MODIS and precip data<br>• Update language about switch to low-intensity sampling (>5 ticks captured in the last 365 days, vs. 1 or more ticks captured in the last 365 days)<br>• Remove requirement to count ticks (this will be done by the taxonomist)<br>• Remove requirement to ship ticks on ice packs |
| L | 02/01/2022 | ECO-06692 | • Updated to new template (NEON.DOC.050006 REV K)<br>• Update time-frame and criteria for switching between low and high intensity sampling<br>• Increase scheduling criteria flexibility to be within 2 (high intensity) or 3 (low intensity) weeks of suggested end dates<br>• Weak painter's tape required if collecting larval ticks without forceps. |

| REVISION | DATE | ECO # | DESCRIPTION OF CHANGE |
|---|---|---|---|
| M | 02/12/2026 | ECO-07164 | • Updated to new template (NEON.DOC.050006 Rev M)<br>• Added requirement to collect larvae separately from nymphs and adults<br>• Removed 50mL vials from storage options – require 1.5-10mL flat-bottomed vials<br>• Updated MODIS dates and number of expected bouts in Table 8<br>• Improved descriptions of flagging methods in challenging terrain<br>• Clarified that ground shipping is appropriate even if longer than 3 days |

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# 1 OVERVIEW

## 1.1 Background

Ticks transmit numerous pathogens that infect wildlife, livestock, and humans, including the etiological agent of Lyme disease (*Borrelia burgdorferi*), the most frequently reported vector-borne disease of humans in the United States. Among arthropod vectors, ticks are particularly sensitive to meteorological conditions and associated physiological constraints (e.g., Eisen et al. 2016, Ogden et al. 2018), making it highly likely that the demography and biogeography of many tick species, and the pathogens they transmit, will be affected by climate change.

Further, the multi-host lifecycles of most tick species increase their ecological connectivity and sensitivity to community-level perturbations that may arise from changes in human land- and resource-use practices. Based on these epidemiological and ecological characteristics, ticks and tick-borne pathogens are an important component of the NEON Terrestrial Observation System. The objectives of sampling are to quantify spatio-temporal changes in the abundance of ticks at NEON sites and in the presence of infection by associated tick-borne pathogens. Rationale for the sampling protocol provided in this document can be found in the NEON Science Design for Vectors and Pathogens (AD[05]).

## 1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. Documentation of content changes (i.e., changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

### 1.2.1 NEON Science Requirements and Data Products

This protocol fulfills Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products and are documented in the NEON Scientific Data Products Catalog (RD[03]).

# 2 RELATED DOCUMENTS AND ACRONYMS

## 2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

| | | |
|---|---|---|
| AD[01] | NEON.DOC.004300 | EHS Safety Policy and Program Manual |
| AD[02] | NEON.DOC.004316 | Operations Field Safety and Security Plan |
| AD[03] | NEON.DOC.000724 | Domain Chemical Hygiene Plan and Biosafety Manual |
| AD[04] | NEON.DOC.050005 | Field Operations Job Instruction Training Plan |
| AD[05] | NEON.DOC.000911 | NEON Science Design for Vectors and Pathogens |
| AD[06] | NEON.DOC.004104 | NEON Science Data Quality Plan |

## 2.2 Reference Documents

Reference documents contain information that supports or complements the current document. Examples include related protocols, datasheets, or general-information references.

| | | |
|---|---|---|
| RD[01] | NEON.DOC.000008 | NEON Acronym List |
| RD[02] | NEON.DOC.000243 | NEON Glossary of Terms |
| RD[03] | NEON.DOC.002652 | NEON Data Products Catalog |
| RD[04] | NEON.DOC.001271 | OS Protocol and Procedure: DMP – Data Management |
| RD[05] | NEON.DOC.001583 | Datasheets for TOS Protocol and Procedure: Tick and Tick-Borne Pathogen Sampling |
| RD[06] | NEON.DOC.000793 | Tick Drag Cloth Assembly Procedure |
| RD[07] | Available via download of data from NEON portal | NEON Raw Data Ingest Workbook for TOS Tick Abundance, Diversity, and Pathogen-status |
| RD[08] | NEON.DOC.003282 | NEON Protocol and Procedure: SIM – Site Management and Disturbance Data Collection |
| RD[09] | NEON.DOC.005247 | AOS/TOS Standard Operating Procedure: NEON Aquatic and Terrestrial Site Navigation |
| RD[010] | NEON.DOC.005224 | NEON Protocol and Procedure: SCS – Shipping Ecological Samples and Equipment |

## 2.3 Acronyms

All acronyms used in this document are defined in RD[01].

## 2.4 Definitions

**Fulcrum**: Software tool used to create NEON electronic data entry applications.

**ServiceNow**: Software tool used for problem/incident tracking and resolution.

## 3 METHOD

Tick and tick-borne pathogen sampling involves the collection of ticks along the border of a 40m x 40m plot using drag and/or flag sampling (**Figure 1**). Following minimal in-house processing, samples are sent to one or more external facilities where ticks are enumerated and identified to the lowest taxonomic rank possible (preferably species). A subset of identified ticks is tested to quantify the prevalence of infection by various pathogens. Some ticks are sent to the NEON Biorepository for long-term archiving.

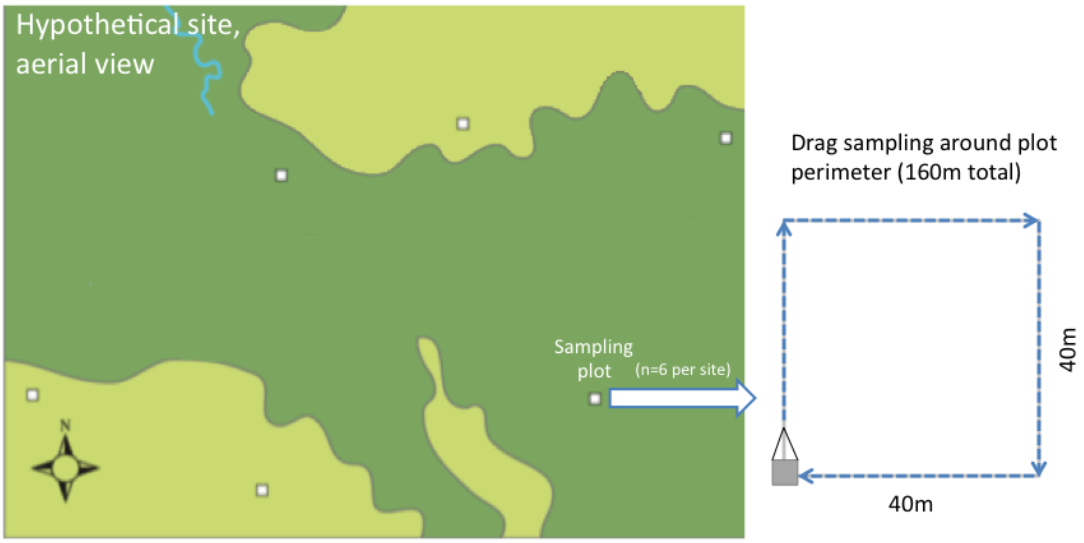


**Figure 1**. A generic site layout for tick sampling along the 40 x 40 m border of the six select tick plots within each field site.

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field technicians **must** follow the protocol and associated SOPs. Use NEON's problem reporting system to resolve any field issues associated with implementing this protocol.

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. It is therefore essential that field personnel carry out this protocol as outlined in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that technicians document the problem and enter it in NEON's problem tracking system.

Quality assurance is performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[06]).

# 4 SAMPLING SCHEDULE

## 4.1 Sampling Frequency and Timing

At each site, a bout of sampling occurs at six Distributed plots. Sampling frequency varies according to whether or not ticks have been detected. **A low intensity schedule** involves one bout every six weeks, while a **high intensity schedule** involves one bout every three weeks (**Table 1**). Sites that are sampling at low intensity will move to high intensity in the following season if more than 5 ticks were collected in the preceding field season at the site level. Sites that are sampling at high intensity will only revert to low intensity if 5 or fewer ticks per season were collected at the site in all of the 5 previous field seasons. If more than 5 ticks are collected per season at a site in any of the 5 previous field seasons the site will remain at high intensity for the next five-year interval, even if some field seasons saw the collection of fewer than 5 ticks. In summary:

To switch from low- to high-intensity sampling:

- More than 5 ticks were collected in the preceding field season at the site level.

To switch from high- to low-intensity sampling:

- All of the previous 5 field seasons saw collection of 5 or fewer ticks per season.

Field ecologists are responsible for monitoring their data annually to determine whether sites need to switch their sampling intensity for the upcoming field season. The tick detection datasheet (NEON.DOC.001583) provides a mechanism for sites sampling on the low intensity schedule to assess whether enough ticks have been collected to trigger high intensity collection. The TCK Field Sampling Application in Fulcrum can also be used to track tick counts at low intensity sites. Selection from the possible drop-down values of: 1, 2, 3, 4, 5, or >5 will be required for entering tick counts when target taxa are present, and the low intensity site button is selected.

Once high intensity sampling is initiated at a site, it continues for five years. The data should be reviewed by field ecologists on an annual basis, and if more than five ticks have been collected in any of the previous five years, the site will remain at high intensity. If a site reverts to low intensity sampling, it will resume high intensity sampling the field season following the season when more than 5 ticks are counted within a season at the site. This design increases the stability of the sampling effort at sites with low but variable tick population sizes, which is helpful for end users as well as for scheduling. To check the count of ticks in the previous five-year interval, identification data should be downloaded from the portal for the appropriate site and years.

**Table 1**. Sampling frequency for Tick and Tick-Borne Pathogen Sampling procedures on a per SOP per plot type basis.

| SOP | Plot Type | Plot Number | Bout Duration | Bouts Per Year | Bout Interval | Yearly Interval | Remarks |
|---|---|---|---|---|---|---|---|
| SOP B | Distributed (K) | 6 | +/- 5 days of scheduled date | Variable, see **Table 7** | Low Intensity: once every 6 weeks<br><br>High intensity: once every 3 weeks | Annual | Sampling must be conducted when the ground is dry.<br>When possible, avoid sampling during the hottest part of the day.<br>Sampling may be delayed in high winds (excess 10-20 mph). |

***Scheduling Considerations***

**Field Work and Laboratory Processing:** After tick specimens are collected from a given Distributed plot, the specimens should be:

- Held in vials containing 95% ethanol and stored at 25°C to retain their integrity. It is important that the level of ethanol completely covers all specimens for proper preservation.

### 4.2 Criteria for Determining Onset and Cessation of Sampling

Bouts of tick and tick-borne pathogen sampling are conducted annually within the site-specific sampling window (**Table 7**). The start and end of sampling each season should coincide with key phenological milestones, with sampling beginning within two weeks of the onset of green-up and ending within two weeks of dormancy/senescence. The sampling windows prescribed in **Table 7** represent the average timing for these events by site. Sampling should be scheduled to begin within one week of the indicated start date (one week earlier is acceptable) and complete no sooner than two weeks (high intensity sites) or three weeks (low intensity sites) before the indicated end date. If green-up or dormancy at a site differs by more than one month from the listed estimated dates below for two subsequent years, issue a problem ticket to NEON Science. Note that for scheduling purposes, the guidance around starting and stopping sampling within a certain distance of the MODIS-defined start and end dates should take precedence over the expected number of bouts listed in **Table 7**. Do not cancel bouts that are within the window in an attempt to meet the estimated number of expected bouts.

**Temperature threshold**: For both the high and low intensity sampling schedules, a bout of sampling will only be performed if the high temperature on two consecutive days prior to planned sampling was > 0°C. Obtain meteorological data based on sensors located as close as possible to the sampling site.
**Partial plot sampling or incomplete bouts**: A standard and complete tick bout samples 160 $m^2$ at each of six plots per site and occurs in a single day. However, problems may arise during the bout that result

in fewer than six plots sampled in a day (e.g., only four of six plots completed) or significantly less than 160 meters sampled at a given plot (e.g., 60 meters sampled at plot CPER_001).

**Incomplete bouts due to inclement weather or safety concerns**: Bouts may be completed on a subsequent day and should follow **Table 2** when determining allowable delays and needed reporting.

- For example: Ticks are scheduled for sampling on March 4th at SRER; domain staff decide to conduct the bout on March 8th because the weather forecast is rainy earlier in the week. On March 8th, 5 plots are completely sampled, but the last plot cannot be sampled due to weather conditions. The last plot is sampled March 11th (within 5 days of the first plot sampled, and within the 21 day delay window for a low-intensity site; see **Table 2**) and a Service Now incident is made to document the delay (per **Table 3**).
- A plot must be sampled at least 80 meters to count as a sampled plot.

If less than 80 meters are sampled at a given plot due to inclement weather or safety concerns, the plot may be reattempted on a subsequent day. Follow **Table 3** when determining allowable delays and needed reporting. No data entry should be made for these partial plots and any ticks collected from partial plots should be discarded. For the tick protocol, data are deleted when the minimum sampling distance is not met because of the highly aggregated nature of tick distributions. Retaining samples collected when less than the minimum length of dragging/flagging was completed can result in skewed abundance estimates.

- For example: Ticks are scheduled for sampling on March 4th at SRER; domain staff decide to conduct the bout on March 5th because the weather forecast is cold earlier in the week. On March 5th, sampling begins at the first plot SRER_001 but is terminated due to safety concerns after sampling 40 meters of the transect. No data entry will be made for this partial plot and any ticks collected at the plot are discarded. On March 6th the team reattempts the bout, successfully conducts the entire 160 $m^2$ drag for plot SRER_001, and completes normal sampling at the other 5 plots. No Service Now incident is needed because the bout was completed within the 5-day window for the bout.

### 4.3 Timing for Laboratory Processing and Analysis

Tick samples held in vials containing 95% ethanol and stored at 25°C will retain their integrity as long as the level of ethanol completely covers all specimens. Ideally, samples should be sent to the identification facility within 3 months of collection to enable publication of the data on the NEON data portal prior to the following field season. Domain Support Facilities should follow their domain-specific shipping schedule when sending ticks for identification and enumeration (see SOP E: Sample Shipment).

### 4.4 Sampling Timing Contingencies

Before the field season begins, all sampling bouts for the year are scheduled in advance in accordance with the dates listed in Appendix C and sampling intensity of the site (low-intensity vs. high intensity

sample schedule). During the season, Field Operations may shift the date of sampling 5 days forward or backward from the planned sampling date if weather or site management would otherwise prevent sampling on the originally scheduled sampling date. Shifting the schedule is at Field Science discretion but requires that staff are available to perform sampling on the alternate date. If a bout is shifted for this reason, subsequent bouts are expected to be performed on their originally scheduled date.

If **temperature** thresholds (see Section 4.2) are not met and it is not possible to shift the bout +/- 5 days (as described above), the bout may be further delayed or canceled according to the directions in **Table 2**. Sampling should occur at the next scheduled bout when temperature thresholds are met.

If the **sampling conditions** below are not met and it is not possible to shift the bout +/- 5 days, the bout may be further delayed or canceled according to **Table 2**.

- Sampling should be conducted when the ground is dry. Do not sample if the ground is moist enough to saturate the cloth with water (e.g., heavy morning dew, following a rain event, or in snow). It is permissible to sample as long as the drag cloth is not becoming drenched all the way to the center (e.g., the edges are slightly damp to the touch).
- If possible, avoid sampling during the hottest part of the day (mid to late afternoon) on days for which the high temperature is at or near the annual high temperature for the site.
- Sampling may be delayed in high wind conditions (in excess of 10-20 mph) where winds disrupt appropriate execution of tick sample protocols.

Occasionally, inappropriate sampling conditions or low temperatures may occur during the **last scheduled bout** of the season and delayed implementation (up to 10 days for high-intensity sites, up to 21 days for low-intensity sites) would result in conducting the bout after the estimated end date provided in **Table 7**. The dates in **Table 7** are only a scheduling guide; it is permissible to conduct sampling after that date when accommodating a delay so long as the temperature and sampling requirements are met when the bout is conducted.

**Table 2**. Contingency decisions for low-intensity and high-intensity Tick and Tick-Borne Pathogen Sampling Protocol.

| Delay/Situation | Action | Outcome for Data Products |
|---|---|---|
| Bout +/- 5 days of scheduled date | Ideally, sampling occurs on the day it is scheduled per the master schedule. However, flexibility is allowed only if a bout were otherwise impossible to conduct on the scheduled date (e.g., weather conditions, managed burns, etc.)<br>**No documentation needed** in Service Now. | Slight increases in temporal variability/inconsistency in time series data. |

| Delay/Situation | Action | Outcome for Data Products |
|---|---|---|
| 5 days < delay ≤ 10 days (high intensity) or 21 days (low intensity) of original scheduled date | If the delay occurs prior to the start of or during the sampling bout, and the issue(s) causing the delay affects all plots at the site, reattempt the ***entire*** bout at the conclusion of the delay. **Submit a problem ticket for delays exceeding five days.**<br>Do not push back dates for subsequent sampling bouts. If the issue(s) causing the delay does not affect all plots at the site, conduct sampling at the plots that are not affected by the delay and submit a problem ticket to NEON Science for guidance about sampling at affected plots. | Larger increases in temporal variability/inconsistency in time series data. |
| Delay > 10 days (high intensity) or 21 days (low intensity) of original scheduled date | If the delay occurs prior to the start of or during the sampling bout, and the issue(s) causing the delay affects all plots at the site, **cancel the sampling bout and submit a problem ticket**.<br>Do not push back dates for subsequent sampling bouts. If the issue(s) causing the delay does not affect all plots at the site, conduct sampling at the plots that are not affected by the delay and submit a problem ticket to NEON Science for guidance about sampling at affected plots. | |
| Delays ≤ 10 days (high intensity) or 21 days (low intensity) that occur at the end of the field season and push the sampling date past the dates listed in Appendix D | At the end of the season, a delay may push a scheduled bout beyond the estimated end dates specified in Appendix C<br>The bout may still be attempted after the date listed in Appendix C **IF** sampling conditions and temperature requirements are met. | Moderate increases in temporal variability/inconsistency in time series data due to the delay. |

### 4.5 Missed or Incomplete Sampling

Sampling according to the schedule is not always possible, and multiple factors may impede work in the field at one or more plots or sampling locations in a given bout. For example:

- Logistics – e.g., insufficient staff or equipment
- Environment – e.g., deep snow, flooding, or inclement weather
- Management activities – e.g., controlled burns, pesticide application

Instances such as those listed above must be documented for scheduling, tracking long-term plot suitability, and informing end users of NEON data availability. Some types of missed sampling are due to

events that should be recorded in the Site Management App; refer to the Site Management and Event Reporting Protocol for more detail (RD[08]).

**Missed or Incomplete Sampling Terms**

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates**: Bout-specific sampling dates (**Table 2**).
- **Scheduled Sampling Dates**: Bout-specific sampling dates scheduled by Field Science and approved by Science. These dates coincide with or are a subset of the Protocol Sampling Dates.
- **Missed Sampling**: Incidence of *scheduled sampling* that did not occur. Missed Sampling is recorded at the same resolution as data that are ordinarily recorded.
- **Sampling Impractical**: The field name associated with a controlled list of values that is included in the data product to explain a Missed Sampling event – i.e., why sampling did not occur.
- **Rescheduled**: Missed Sampling is rescheduled for another time according to one of the scenarios documented in **Figure 2**, resulting in no change to the total number of sampling events per year.

The documentation that must accompany missed sampling depends on the timing, subsequent action, and the audience appropriate for numerous scenarios (**Figure 2**).

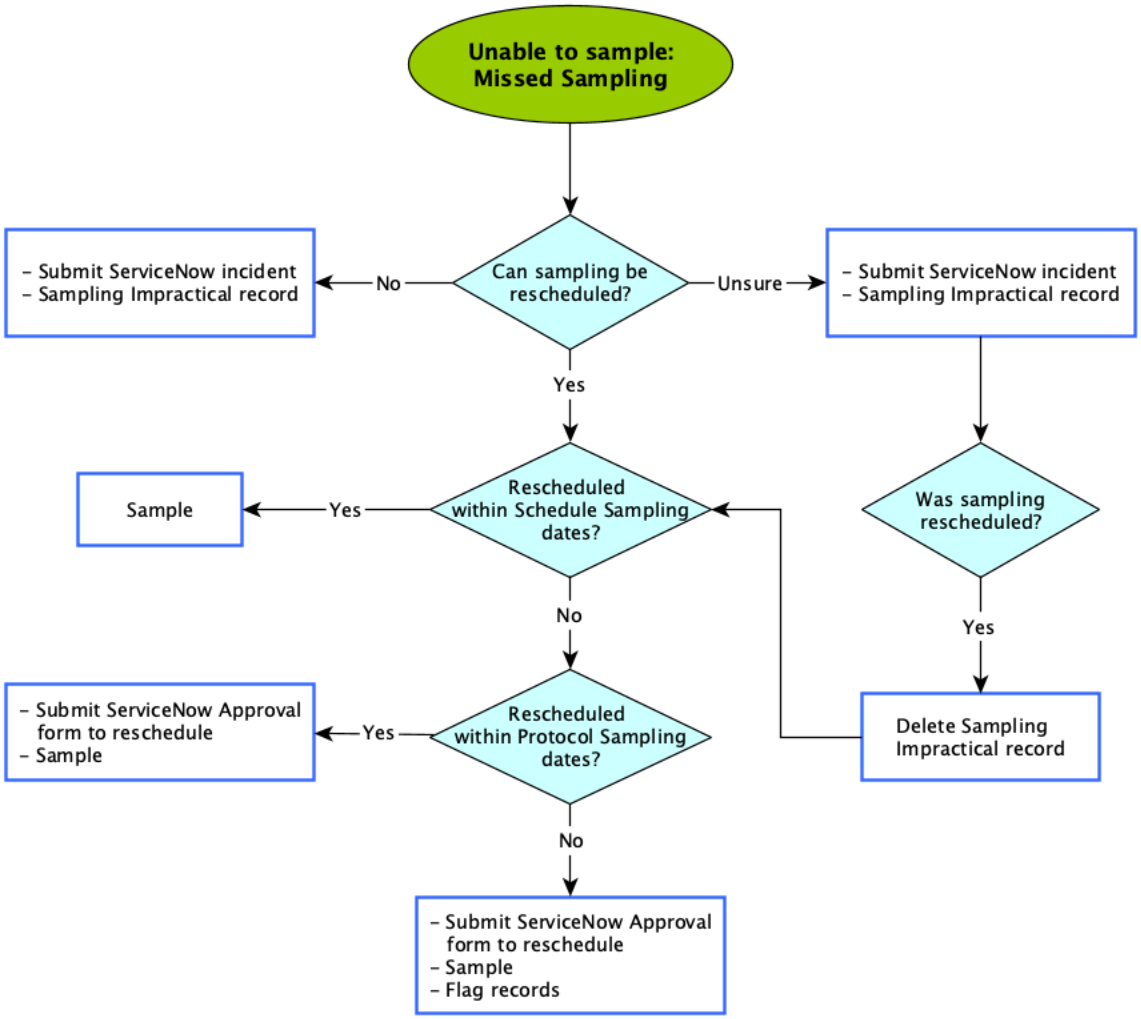


**Figure 2**. The documentation to account for a Missed Sampling event depends on the situation for each sampling unit not sampled per bout that is not sampled. Diamonds represent decision points and boxes describe the required action. Required actions may include: a) Submitting a ServiceNow incident, b) creating a Sampling Impractical record, c) creating a data Flag, d) creating a Site Management record, or e) some combination of (a) – (d).

**To Report Missed or Incomplete Sampling:**

1. Missed or Incomplete Sampling that cannot be rescheduled within the Schedule sampling dates must be communicated to Science by a ServiceNow Incident.
    - For Missed Sampling that is Rescheduled, there are some cases that require approval by Science and Operations (**Figure 2**).
    - Consult **Table 3** below to determine required actions if scheduled activities are delayed or canceled.

2. Create a Fulcrum record for each Missed Sampling event in the field that cannot be rescheduled. That is, if data are recorded in the field at the plot level, a record must be made for each plot missed.
    a. Record each plot not sampled in each bout; it could be all plots or a subset of plots.
3. For each Missed Sampling record, the **Sampling Impractical** field must be populated in the mobile collection device (**Table 4**).
4. For Rescheduled sampling events that occur outside of the defined Protocol Sampling Dates and for which plant dormancy has largely occurred, a protocol-specific Flag within the field called biophysicalCriteria must also be recorded (**Figure 2**).
    a. biophysicalCriteria should have an entry of conditions not met: most plants senesced if sampling is scheduled to occur before plant green-up, or after the plants have senesced for the season. Otherwise, this field will default to OK – no known exceptions

**Table 3**. Guidance for responding to delays and cancellations encountered during implementation of the Tick and Tick-Borne Pathogen Sampling protocol.

| Activity Name | Days Delayed from Schedule | Delay Action | Cancellation Action |
|---|---|---|---|
| TCK Field Sampling | Bout +/- 5 days of scheduled date | No action needed | No action needed |
| TCK Field Sampling | 5 days < delay ≤ 10 (high intensity) or 21 (low intensity) days of original scheduled date | Submit problem ticket for delays > 5 days | Submit problem ticket – Notify Science for guidance |
| TCK Field Sampling | Delay > 10 (high intensity) or 21 (low intensity) days of original scheduled date | Submit problem ticket | Cancel bout |
| TCK Field Sampling | Delays ≤ 10 (high intensity) or 21 (low intensity) days that occur at the end of the field season and push the sampling date past the dates listed in Appendix C. | Submit problem ticket for delays > 5 days | Submit problem ticket – Notify Science for guidance. If sampling is rescheduled after plants have senesced, use **biophysicalCriteria** flag to indicate conditions not met: most plants senesced. |

**Table 4**. Protocol-specific Sampling Impractical reasons entered in the Fulcrum application. In the event that more than one is applicable, choose the dominant reason sampling was missed.

| Sampling Impractical reason | Description |
|---|---|
| Temperature low | Ambient temperature lower than requirements specified in protocol |
| Location wet | Location wet |
| Location flooded | Location flooded |
| Logistical | Site or plot access compromised, staffing issues, errors (e.g., equipment not available in the field) |
| Management | Management activities such as controlled burn, pesticide applications, etc. |
| Location snow covered | Location snow covered |
| Location frozen | Location frozen |
| Other | Sampling location inaccessible due to other ecological reason described in the remarks |
| Too windy | Excess of 10-20 mph winds |
| Location vulnerable to planned sampling | Location vulnerable to excessive technician impacts during planned sampling |

### 4.6 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

An experienced two-person team can complete sampling of ticks at a single plot in approximately 30 to 120 minutes (see **Table 5**). This entails dragging/flagging around the perimeter of the plot and transferring ticks into one or more sample vials.

**Table 5**. Estimated staff and labor hours required for implementation of the Tick and Tick-Borne Pathogen Sampling protocol.

| SOP | Estimated time | Suggested staff | Total person hours |
|---|---|---|---|
| SOP A: Preparing for sampling | 0.5 hr/bout | 1 | 1 hr/bout |
| SOP B: Field Sampling | 0.25 – 2 hrs/plot | 2 | 0.5-4 hrs/plot |
| SOP C: Post-Field Sampling Tasks | 0.5-3 hrs/bout | 1 | 0.5-3 hrs/bout |
| SOP D: Data entry and verification; QAQC | 1 hr/bout | 1 | 1 hr/bout |
| SOP E: Sample shipment | 0.5 hr/bout | 1 | 0.5 hr/bout |

## 5 SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the EHS Safety Policy and Program Manual (AD[01]) and Operations Field Safety and Security Plan (AD[02]). Additional safety issues associated with this field procedure are outlined below. If an employee witnesses any unsafe conditions or uncontrolled hazards that present an imminent danger, they should immediately take action to stop work and report such conditions to their manager. Employees must also report all workplace injuries, illnesses, incidents, or chemical/reagent releases to the environment as soon as possible, regardless of the severity.

Field personnel are collecting biting arthropods, but there is no increased risk of infection by zoonotic pathogens during implementation of this protocol than in general fieldwork. We recommend that field personnel wear light-colored clothing when implementing this protocol to improve visibility of ticks on clothing prior to and following sampling. In addition, it is highly recommended to tuck pants into rubber knee high boots, or tuck pants into socks. Follow guidelines provided in the Operations Field Safety and Security Plan (AD [02]) to prevent tick bites and take appropriate action if an embedded tick is found. Personnel working with ticks should familiarize themselves with the Zoonotic Diseases section of AD [02].

**IMPORTANT:** Use of insect repellent is highly recommended, but application is left as a personal safety choice. If used, insect repellent must be applied at least **30 minutes prior** to arriving in the field. If applying insect repellent in spray form, DO NOT apply in the vicinity of sampling equipment. After applying insect repellent, clean the palms of hands (e.g., with soap/water or alcohol-free hand wipes) before handling any sampling equipment. Both permethrin (0.5%) and DEET (up to 40%) are excellent repellents and can be used to treat field clothes well in advance of field sampling (two to four hours prior). Application of insect repellent *less* than 30 minutes before sampling will reduce tick sampling success and data quality; thus, it is important that use of these products occur before arriving in the field.

When surveying in areas where *Toxicodendron spp.* (e.g., Poison Ivy) are present, the use of Technu is recommended after sampling. Staff should also consider wearing gloves to avoid contact with oils that may be on the drag cloth.

This protocol does require the use of chemicals (Ethanol). Safety Data Sheets (SDS) shall be readily available for review whenever chemicals are being transported or used during this activity.

# 6 PERSONNEL

## 6.1 Training Requirements

All technicians must complete protocol-specific training as required in the Field Operations Job Instruction Training Plan (AD[04]). Additionally, technicians must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[05]).

## 6.2 Specialized Skills

Prior experience collecting ticks or conducting entomological fieldwork is desirable but not required. Personnel should have good fine manual coordination for handling individual specimens.

# 7 STANDARD OPERATING PROCEDURES

## SOP Overview

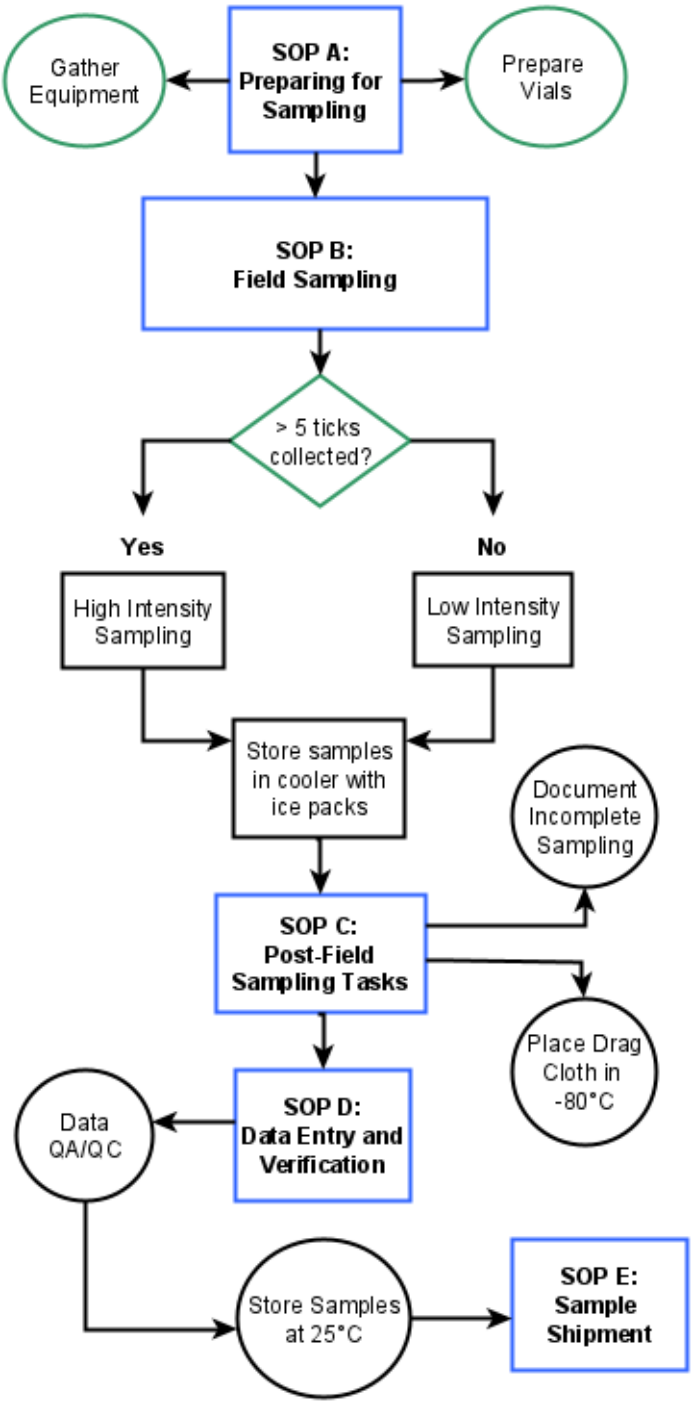


**Figure 3**. A high level workflow diagram that visually shows how the separate SOPs are sequentially connected.

- **SOP A**: **Preparing for Sampling.** Sample vials are prepared, and equipment is gathered.
- **SOP B: Field Sampling.** Tick collection is completed using dragging or flagging methods.
- **SOP C: Post-Field Sampling Tasks.** Samples are stored, cloth is cleaned, and incomplete sampling is documented.
- **SOP D: Data Entry and Verification.** All data are quality checked.
- **SOP E: Sample Shipment.** Samples are shipped to the external taxonomic laboratory.

## SOP A Preparing for Sampling

### A.1 Preparing for Data Capture

Mobile applications are the preferred mechanism for data entry. Mobile devices should be fully charged at the beginning of each field day, whenever possible.

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times.

### A.2 Preparing for Field Sampling

At least one week prior to a sampling bout:

1. Plan and save sampling routes for field teams using standard site navigation procedures (RD[07]). Route planning enhances sampling efficiency and helps avoid accidental foot traffic within NEON plots. Evaluate current plot conditions according to the criteria in Section 4.2.
2. Print out datasheet(s) on waterproof paper.
3. Be sure reusable ice packs (0°C) are frozen.

### A.3 Labels and Identifiers

Many protocols use a mix of human intelligible labels and barcodes in their workflow. Barcodes are useful for minimizing transcription errors and tracking samples from the domain support facility to external locations. **All field samples must use a barcode and all samples being shipped from the DSF must have a barcode.**

1. In general, apply a barcode if a sample is generated and subsequently processed over a period of > 5 business days.
2. Barcodes are optional for temporary samples that are created and consumed in < 1 day. For example, a 50 mL vial containing ethanol and larvae on tape that will be transferred to another vial does not need a barcode.

Best practice for barcode application and maintaining proper adherence to sample containers:

1. All barcodes need to be applied to dry containers for 30 mins before use. Type I (prefix A, plus 11 numbers) are for all field samples and any non-cryo applications; they have a functional temperature range from 4˚C to 105˚C. Type IV (prefix D, plus 11 numbers) accommodates < 10 mL vials.
2. If available, prepare final sample containers by affixing one adhesive barcode label to each vial and/or Ziploc bag used to contain each sample. Type I barcodes are preferred for medium size vials (e.g., 10 mL) or Ziploc bags. Type IV barcodes are **required** for small size vials (less than 10 mL). **Using a Type I barcode on a skinny vial will result in the barcode falling off the vial.**

- Adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 minutes prior, but may be applied at the start of the season).
- Barcode labels should always be oriented such that it is possible to scan them; the scanner will not work on a curved surface. This means aligning the barcode *lengthwise* along a vial, not horizontally wrapping around a vial.
- If your site generates one vial of ticks per tick transect, affix the barcode label to each vial to be filled with a **unique sample**. If multiple vials are required to contain a sample from one transect, place the barcode on the Ziploc bag that will contain all vials associated with that sample.

  *Example*: A sample collection from OSBS_005 fills one 10-mL tube. The single barcode is applied to the sample vial.

  *Example*: A sample collection from OSBS_005 fills ten 10-mL tubes. The single barcode is applied to the Ziploc container that contains all ten vials, *not* each vial containing 1/10 the sample.

  *Note:* Neither the data entry mechanism nor our database can handle 10 barcodes mapping to the same sample. Barcodes are unique, but are not initially associated with a particular sample, so you are encouraged to make these up in advance.

3. Prepare collection sample vials by affixing both external sampleID label and the barcode label (**Table 6**). The sampleID (**Figure 5**) is the plotID and the date (YYYYMMDD), separated by a period. As an example, the sampleID "OSBS_002.20130802" would indicate that the labeled vial contains ticks collected in plot 002 at Ordway Swisher Biological Station on August 2, 2013.
   - External labels: External sampleID labels may be legibly written directly on the vial with ethanol-safe permanent marker or pre-printed on adhesive labels (preferred). Labels should be oriented with the beginning of the sampleID towards the vial opening.
   - Labeling in the field: If temporary labels are added to vials in the field, be sure that vials have both barcode and external labels before shipping samples.


**Figure 4**. An example of a Type I (left) and Type IV barcode (right). Type I barcodes are larger in size, field-tolerant barcodes with a prefix of 'A' followed by 11 numbers. Type IV barcodes are smaller in size with a prefix of 'D' followed by 11 numbers.

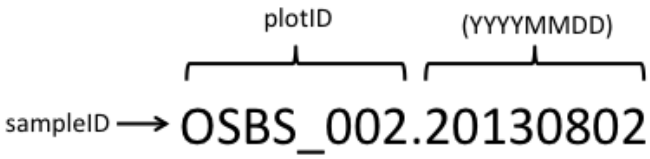


**Figure 5**. Annotated sampleID example.

### About Barcode Uses and Placement

Although it is always acceptable to use barcodes, in some cases barcodes are absolutely required. Barcodes (Type I and Type IV) should be aligned lengthwise along each vial.

**Table 6**. Barcode requirements for sample types generated by the Tick and Tick-Borne Pathogen Sampling protocol. The rule of thumb is that the primary field sample will ALWAYS need a barcode due to its importance in generating future samples. Likewise, the final disposition of all vialed samples must have a barcode affixed to assist in the shipping and receipt of samples destined for the Biorepository or an external laboratory.

| Sample Type | Description | Example Identifier | Fulcrum App | Container Type | Barcode Used | Barcode Required? | Barcode Qty |
|---|---|---|---|---|---|---|---|
| Field samples | Ticks collected per plot | CPER_001.20180904 (sampleID_plotID.collectDate) | TCK: Field Sampling | 10 mL vial | Type I | **Always Required** | 1 per plot |
| Field samples | Ticks collected per plot | CPER_001.20180904 (sampleID_plotID.collectDate) | TCK: Field Sampling | < 10 mL vials | Type IV | **Always Required** | 1 per plot |
| Field samples | Ticks collected per plot | | TCK: Field Sampling | Ziploc bag | Type I | **Always required** if multiple vials collected in plot | 1 per plot |

### A.4 Immediately Prior to Field Sampling

1. Gather all necessary equipment for field sampling.
2. Fill sample vials ¾ full with 95% ethanol.
3. Insect repellent should be applied at least thirty minutes prior to sampling. If using insect repellent in spray form, do not apply in the vicinity of sampling equipment. After applying insect repellent, clean the palms of hands (e.g., with soap/water or alcohol-free wet wipes) before handling any sampling equipment.
    - Application of repellent less than 30 minutes before sampling will reduce sampling success, so ensure that all application occurs at least 30 minutes ahead of time.
4. Use the checklist (Appendix B) to ensure that all required materials are in the field truck prior to sampling.

## SOP B Field Sampling

Sample using one or both of the two sampling methods. Drag sampling (SOP B.2) is the preferred method used for tick collection. Flagging (SOP B.3) is used as a substitute for dragging when vegetation is too thick to allow the drag cloth to be pulled along the ground.

- The Fulcrum App used for this protocol is: TCK Field Sampling [PROD]. A link to the Fulcrum Manuals can be found in the SSL.

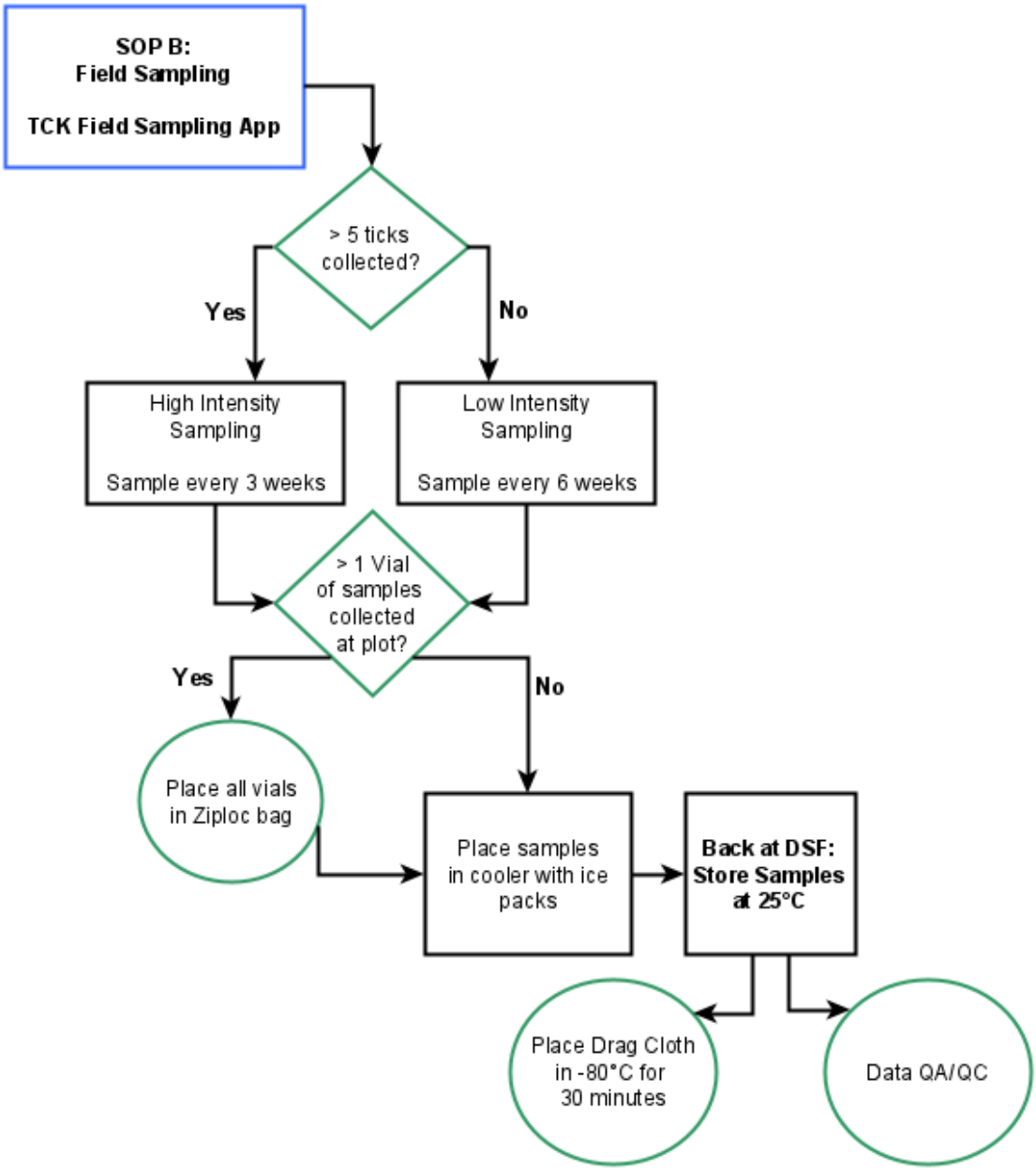


**Figure 6**. An expanded diagram of the field sampling workflow (SOP B).

### B.1 About Sampling Transects

1. Sample along a fixed path that follows the shortest straight-line distance between plot corners and thus covers the full perimeter of the plot (**Figure 7**). Sampling may proceed in either a clockwise or counterclockwise direction. Ideally, 160 meters is sampled per plot (four 40 meter transects).
    a. Always record the total horizontal distance sampled, with a target accuracy of +/-2 meters.
    b. Try to maintain as narrow and consistent a path as possible to minimize trampling surrounding vegetation. Should a path become worn, you may need to drag the cloth beside the path, just inside the plot perimeter.
2. If straight-line transects are not possible, choose an alternative path that minimizes detours from the original perimeter, while staying within the range of acceptable sampling distance (minimum 80 meters, maximum 180 meters). If the distance covered falls out of the range of accepted sampling distance, issue a problem ticket.
    a. If a large obstacle (e.g., rock, tree, cluster of shrubs) is present along the transect, divert the sampling path as little as possible into the plot. Be sure that all diversions are directed into the plot, rather than outside the plot boundaries.
    b. If the obstacle is too large to divert around, sample up to the obstacle, pick up the cloth, then begin sampling again on the other side of the obstacle.
        i. *Example:* If a narrow creek runs down the middle of the plot, simply step over or cross the creek where it crosses the sampling transect.
3. Flagging landmarks may help maintain consistency across bouts in transect detours.

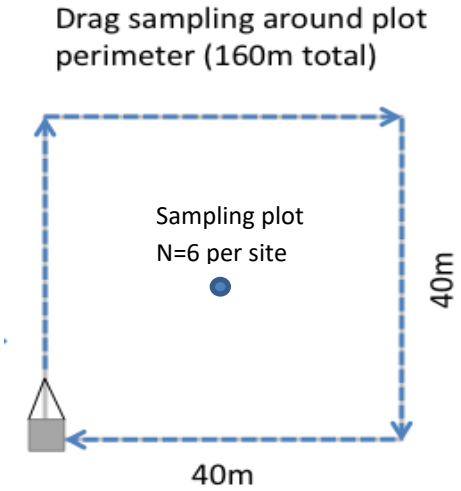


**Figure 7**. Schematic of sample and plot transects.

### B.2 Overview of Drag Sampling Method and Frequency of Checks

1. Place the drag cloth on the ground. One member of the two-person team should pull the cloth while the other walks behind the cloth. The team member walking behind the cloth must ensure that the cloth does not flip over, get bunched up, or become caught on plants or rocks while being pulled along the ground.
2. Drag the cloth at a pace that is slow and steady.
    a. Qualitatively, this pace is equivalent to a leisurely stroll (think wedding procession). Slowly counting "1 Mississippi" for each step forward is a good approximation of appropriate cadence. When measured on a grass soccer field, it took ~50 seconds to drag 15 meters at the proper pace.
3. Ensure that the entire cloth stays in contact with the ground or vegetation.
    a. When pulling the cloth, make sure there is enough pull cord between the individual pulling the cloth and the cloth itself so that **the leading edge of the cloth stays as flat as possible on the ground.** Too little pull cord between the person and the cloth will cause the leading edge of the drag cloth to rise up and lose contact with the ground.
    b. Weights may be attached to the edges of the cloth as necessary if conditions are windy. Note that the weights are not intended to hold the cloth down in the absence of wind. Under calm conditions, the downward pull of gravity on the cloth is acceptable to keep the cloth in contact with the ground.
4. Stop to collect ticks every 5-10 meters as described in SOP B.5.

### B.3 Overview of Flag Sampling Method and Frequency of Checks

1. The flagging cloth is a modified drag cloth: unclip the drag cloth pull cord and any attached weights from the drag cloth.
2. To sample, hold the drag cloth by one end of the wooden dowel. Gently "wave" the flag, guiding it over a sampled area. This movement and manner of holding the cloth allow greater precision to move it over/around/beneath vegetation.
3. Periodically crouch down and sweep the flag underneath vegetation. While the cloth can be passed over and around vegetation, sampling the ground underneath vegetation will ensure that flagging is most comparable to dragging.
    - Do not attempt to flag spiny/thorny vegetation (e.g., brambles, cacti) as this will damage the cloth. Instead, drag underneath this vegetation if you can avoid catching the cloth on spines/thorns.
    - If the vegetation is low growing and it is not possible to pass the cloth underneath, consider the vegetation an obstacle (see SOP B.1, Step 2).

- Note that when flagging, especially underneath vegetation, the cloth will generally wrinkle. This will require estimating the total distance sampled with less precision than when dragging.

4. Stop to collect ticks (SOP B.5, Step 6) every 3-4 sweeps, which should be the equivalent of sampling 3-5 square meters.
    - This is with greater frequency than with dragging, as sampling in dense vegetation is more likely to dislodge ticks attached to the cloth.
5. Tips for particularly challenging flagging situations:
    - In successional areas with extremely dense shrubs, it is most important to sample the ground underneath the shrubs since the larger tick-carrying mammals do not tend to travel through dense vegetation. In dense areas, best practice is to crouch as low to the ground as possible to get the flag on the ground where the smaller tick-carrying rodents are most likely to travel.
    - In areas with a high density of downed logs, the optimal flagging strategy is to flag under the log and on both sides. The ticks will not be located on top of the logs, so getting underneath these logs is the most effective strategy.
    - As mentioned above, in thorny areas the flagging should be done underneath and around, rather than on top of the thorns so that they do not tear the cloth. Bare and thorny areas that tend not to yield any ticks are still important to sample. Even if no ticks are encountered, it is important to document that they are not there by completing the sampling. This is because there are some regions of the world where desert-tolerant ticks do occur, and we want to document that they are not present in our sampling areas.

## B.4 When to Use Drag Sampling Versus Flagging

1. Drag sampling is the preferred sampling method since it allows the area sampled to be more accurately quantified. This is important for estimating tick density.
2. During sampling, it is important to try and keep the cloth in direct physical contact with the ground, vegetation, or overlying leaf litter.
    a. When dragging, attempt to make a qualitative assessment of whether the cloth is on or close to these surfaces: is it touching the surface most or all of the time, is it “surfing” up 2-3 inches above the surface as it passes over flexible-stem grasses/forbes, or is it “stilting” 4 or more inches above the surface as it passes over rigid-stemmed shrubs?
    b. The first scenario is ideal for dragging, the second (“surfing”) is acceptable for dragging, and the third scenario (“stilting”) should trigger the use of flagging to keep the cloth closer to the ground. In particular, flagging is an effective means of getting the sampling cloth underneath shrubs and taller/more dense vegetation where small tick carrying rodents are active.

3. If necessary, a combination of dragging and flagging may be used to sample over vegetation types or obstacles. The following are examples of scenarios that may be encountered:

    - **Low stature grass, herbs, or leaf litter**: Sample using dragging or flagging, but the former is preferred since it allows for more accurate quantification of total distance covered during sampling.
    - **Medium to tall grass or herbs, supple vegetation (not woody/rigid):** The cloth might not be in physical contact with the ground, but it can be easily pulled (dragging) or waved/passed (flagging) over the top of the vegetation. Because the vegetation is supple, the weight of the cloth will allow it to be pulled down into the vegetation and closer to the ground by gravity. This can be further accentuated with flagging as the cloth can be pushing down into the vegetation by holding the dowel lower to the ground. In this scenario, sample using either dragging or flagging, but the latter may be preferred when the vegetation is tall because the cloth can be pushed down to a greater degree than by gravity alone.

    - **Medium to tall shrubs, woody vegetation (non-supple) and patchy**: If the vegetation (e.g., woody shrubs) is present at low density such that the drag cloth can be pulled between plants, then use dragging to sample the ground underneath the shrubs. If vegetation density is higher and the drag cloth cannot be pulled between plants, use flagging to sample this interstitial area. If the vegetation is not tall (i.e., ≤ 1 m) you can additionally sample the sides and tops of shrubs using flagging.
    - **Tall woody (non-supple) vegetation**: As with low/medium stature woody vegetation, drag or flag the ground between plants if density is low enough to allow space. If plants are > 1.3 m tall, sample the ground between and underneath plants (i.e., do not sample trunks or woody stems).

### B.5 Tick Collection in the Field

1. Navigate to a consistent corner of the tick sampling plot (usually the SW corner) using maps and/or a handheld GPS. Whatever corner is chosen must be clearly labeled as a tick plot. Verify the identity of the plot location via the plotID labeled on the permanent plot marker (created during plot establishment) or with the alternative marker system required by the site host at sites where the site host does not allow permanent plot markers.
    - Be sure to avoid travel through any portion of the plot, especially the plot perimeter.
2. Perform an inspection of your and your partner's person after arriving at the plot corner and before sampling begins. Remove ticks using duct or masking tape and discard ticks at least 3 m from the transect that will be dragged.
3. Record bout information (personnel conducting the sampling & protocol version) and location information (especially plotID, date of collection and time that sampling was initiated). The fulcrum fields associated with these data are:

- **recordedBy** and **measuredBy**
- **samplingProtocolVersion**
- **siteID** and **plotID**
- **date**, **startTime** and **endTime**

**For each sampling interval, conduct steps 4 through 7:**

4. Begin drag or flag sampling along the perimeter of the plot.
    - If necessary, use a compass to orient along the plot perimeter. If the plot corners are not easily visible between intervals, the person following may remain at the plot corner and provide direction until the next plot corner is located. Flagging landmarks may help.
    - Avoid contact with the cloth. Be aware that ticks may attempt to crawl onto your hands, arms, or body while you inspect the drag cloth.
5. Stop at intervals appropriate to the sampling method and vegetation density to examine the cloth and your person(s) for ticks.
    - Perform a quick scan over the cloth and your person(s) for adult ticks first, as they tend to drop off more quickly than the other life stages.
    - To determine whether an invertebrate is a tick, reference **Figure 8** below. Ticks are small (adults are about the size of an apple seed), but non-tick mites are smaller (less than one mm in size). Adult and nymph ticks have 8 legs, while larvae only have 6.
    - Hold the cloth at arm height to check for ticks. Do not perform a check for ticks with the cloth on the ground as ticks may crawl off or onto the cloth.
    - Scan the cloth in a systematic manner such that you examine the entire cloth on both upper and lower surfaces.
    - Use a hand lens as necessary to distinguish ticks from other arthropods and debris.

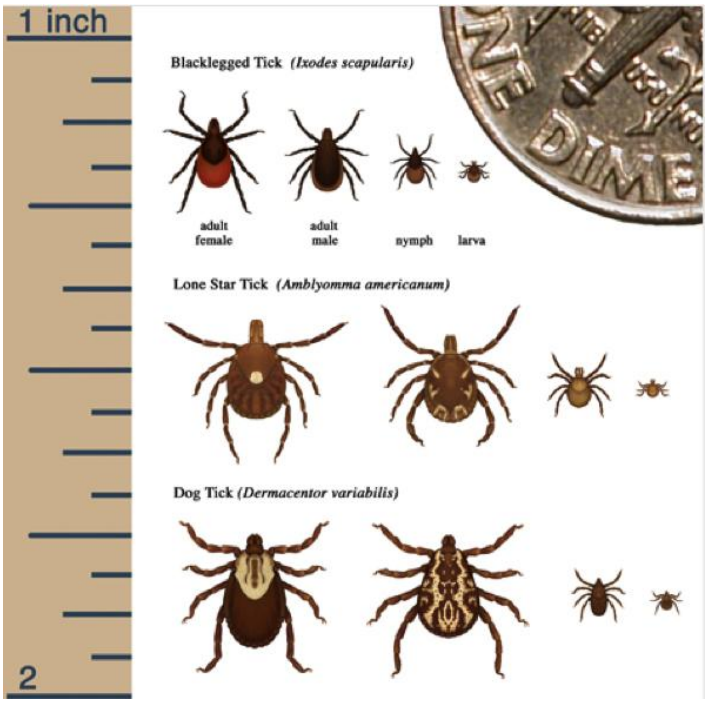


**Figure 8**. Relative sizes of life stages for selected species (courtesy of the Centers for Disease Control and Prevention).

6. Collect ticks and transfer them into the appropriately labeled sample vial containing 95% ethanol. Larval ticks should be stored in a separate vial from nymphs and adults to facilitate identification and enumeration. Record the barcode identifier of the sample vial or Ziploc bag in the mobile application.

    - When handling a tick, use forceps to pick up non-larvae by the leg rather than pinching the body.
    - Additional sample vials can be used if a single vial cannot hold all of the ticks collected during a sampling plot/bout combination, or if both larvae and nymphs/adults are encountered at the plot.
    - If collecting **larvae** with forceps is excessively time-consuming, collect **larvae** with weak painter's tape. This can be affixed to a lint roller to make the tick removal more efficient, but the sticky surface being used to collect the ticks must be the weak painter's tape, not the tape of the lint roller. The weak painter's tape is required to ensure that large larval collections do not become encased in the lint/cotton that is pulled off the cloth by other types of tape.

        i. Remove larval ticks from the drag cloth and your person with weak painter's tape. Do not use duct tape for sampling. Collect as many larvae as possible using as little tape as possible. You should strive to find and remove every larval tick from the cloth.

ii. Completely submerge the tape with ticks in a labeled 50 mL vial (or larger if needed) with 95% ethanol to weaken the tape adhesive. Leave in ethanol for transit back to the lab.

iii. This method should only be used for larvae.

7. If ticks are lost in the field, record this incident using the **sampleCompromised** field as well as a **remark** to describe the situation in more detail. There are two **sampleCompromised** options when ticks have been lost or dropped in the field to indicate whether it is a large or small proportion of the sample. Specifically, these are:

   - Sample Incomplete by > 10% (described in **remarks**). This should be used when a large proportion of the total sample for the plot is lost, which can happen if a vial was misplaced or dropped and spilled.
   - Sample incomplete by < 10% (described in **remarks**). This option should only be selected if there are more than 10 ticks collected at a plot, but one (or less than 10% total) have been dropped or lost. Remarks should include the estimated life stage and other known details about the tick(s) that were lost, if known.

8. Spend no more than 10-20 seconds checking your person(s) for ticks between intervals. Examine areas around the lower legs and feet especially closely. This inspection may be more thorough if done reciprocally (i.e., each team member inspects the other). A more thorough inspection of your person for ticks can be done at the completion of sampling.

9. Continue sampling at appropriate intervals until the 160 m sampling transect is complete. Inspect your person(s) for ticks and add them to the sampling vial.

10. Data should be entered using the mobile data entry application.

    - Enter one record for every plot sampled, documenting end time, meters sampled, and barcode for each sample (if assigned).
    - Be sure to record any notes regarding unusual field conditions that may have affected sampling results. (e.g., cows walking through plot during sampling).
    - Large-scale plot disturbances or site management activities (i.e., burning, mowing, etc.) should be documented in the Site Management and Event Reporting application.

11. Place all labeled samples in an insulated cooler with frozen ice packs for transit back to the lab. While ticks in ethanol do not require a cold chain to be maintained, storage of ticks in extremely hot temperatures (> 30˚C) could compromise sample integrity.

12. After leaving the plot, ticks found on your person(s) should be removed with duct tape and discarded.

### B.6 Sample Storage

When multiple vials are used in the field to contain specimens that correspond to a single **sampleID** (i.e., one collection date at one plot), vials will need to be grouped into a single container (i.e., Ziploc bag, a larger vial). This grouping must be done before samples are stored.

1. If these specimens are all the same life stage and can be consolidated into one vial, transfer all ticks from the same plot and date of collection into a vial that is labelled with a barcode and the appropriate sampleID. Remember that larvae must be kept separately from nymphs and adults. Update the electronic record with the corrected sample barcode and indicate that the number of vials per sampleID is 1. OR
2. If specimens corresponding to the same sampleID cannot be consolidated into one vial, then remove any pre-existing barcode labels from the vials, place these vials into a single bag, and externally label the bag with the sampleID and barcode. If available, you may use a bag that has been pre-labelled with a barcode from SOP A. Update the electronic record with the corrected sample barcode and revise the number of vials per sampleID, if necessary.
3. Upon returning to the lab, repackage any samples as described above and store them at 25°C. A flame fridge rated for flammable material like ethanol is also permissible. Process tick samples within one month of collection. Store samples from the same site/bout combination together (e.g., using a rubber band and/or placing within a resealable bag).

## SOP C Post-Field Sampling Tasks

1. Staff are encouraged to use gloves when working with a cloth that touched a *Toxicodendron* species in the field (i.e., Poison Ivy, Poison Oak, or Poison Sumac).
2. Place the drag cloth in an ultralow (–80°C) freezer for at least 30 minutes to kill any larval ticks attached to the cloth.
3. Either by hand or with duct tape, remove seeds stuck to the drag cloth to prevent introduction to other plots and sites.
4. If the drag cloth is dirty, wash it using fragrance-free laundry detergent, using bleach if necessary, and hang it to dry. If a laundry dryer is used, select a medium heat setting to prevent the drag cloth from shrinking. Always make sure the drag cloth is completely dry and in good condition (i.e., same size as at the beginning of the season, free of holes) before placing in storage.
5. Clean any other equipment as necessary using dilute fragrance-free laundry detergent, dry, and store in a cool, dry place.
6. If vials will be re-used to store specimens, the following protocol should be followed to clean the vials prior to re-use.
    a. The vials and lids should be placed into a sink filled with warm soapy water.
    b. A test tube or bottle brush should be used to give each vial a quick scrub.
    c. Vials and lids should be rinsed in clean tap water followed by an individual rinse with a squirt bottle of DI water.
    d. Vials can be dried on a tray in a drying oven on low temperature overnight if needed. Vials should be stored separately by protocol and site (if possible) for re-use.
7. If larval ticks were collected using the weak painter's tape method, the tape should be retained in ethanol at room temperature for > 1 day to allow the ethanol to weaken the adhesive. Ticks can then be removed into the original collection vial using a paintbrush, pipette or forceps. Be sure to keep the larval ticks separate from any nymphs/adults collected.

### C.1 Document Incomplete Sampling Within a Site

Tick and Tick-Borne Pathogen sampling is scheduled to occur at all prescribed sampling locations according to the frequency and timing described in Section 4 and Appendix C. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory. However, sampling may be shifted from one location to another when sampling is compromised. In general, a sampling location is compromised when sampling becomes so limited that data quality is significantly reduced.

There are two main pathways by which sampling can be compromised. First, sampling locations can become inappropriately suited to answer meaningful biological questions – e.g., a tick sampling plot is compromised after road-building activities. Second, sampling locations may be located in areas that are logistically impossible to sample on a schedule that that is biologically meaningful.

For the tick sampling program, use the following criteria to evaluate each plot:

1. If permanent obstacles are present over 50% or more of the entire perimeter of the plot, then the plot should be proposed for replacement.

    a. Tick sampling should only be conducted in plots in which you are able to sample for ticks (using dragging, flagging, or a combination, as described in SOP B) over 50% or more of the plot perimeter – i.e., sampling occurs over ≥ 80 meters.

    b. Problems arise when woody vegetation is so tall (i.e., > 1 - 1.3 m) and/or dense that a drag cloth cannot easily be pulled over or around the base of plants and flagging in between plants becomes exceedingly time consuming (i.e., > 120 min).

    c. Other obstacles include standing/flowing water, thorns, or wet terrain.

2. If sampling cannot be completed on ≥ 50% of the planned sampling dates over a two-year period, due to temporally variable obstacles or conditions, then the plot should be proposed for replacement.

    a. In some cases, a plot may be acceptable for sampling for a portion of the sampling season and unacceptable for the remainder of the season. For example, large portions of a plot perimeter may be wet early in the sampling season but dry out later.

    b. Alternatively, large portions of the plot perimeter may be associated with supple, low growing vegetation early in the sampling season that becomes tall/dense/woody later in the season.

    c. You will need to use local knowledge to estimate the portion of the year that cannot be sampled, and one or two field seasons may be required to quantify this with confidence for questionable plots. Over the long term, each accepted plot needs to support sampling of scheduled bouts ≥ 50% of the time.

3. Representative cover type: When inclined to reject a plot based on obstacles, consider whether the conditions are unique to this plot or are typical of plots within this vegetation type.
    a. If the plot is not representative, then rejecting the plot and evaluating alternative plots in the same vegetation type is advisable.
    b. Alternatively, if all of the plots in the vegetation type are likely to be characterized by these features (e.g., all of the woody wetland plots are too wet, or the plant density in all of the shrub plots is too high), then issue a problem ticket. Reallocating plots to one or more other vegetation types at the site may be considered.

If sampling at a given plot is not possible during a given bout a problem ticket should be submitted by Field Science staff.

**To document locations not sampled during the current bout:**

1. Review the completed sampling effort and create **Sampling Impractical** records as described in Section 4.5 for plots at which sampling was scheduled but was not completed.
2. To document whether a location is compromised according to the criteria above:
    a. Review **Sampling Impractical** records from the TCK: Field Sampling[PROD] application and Portal data to identify locations where sampling was scheduled but was not completed due to environmental or site management factors.
3. Create an incident with the following naming convention to document the missed sampling and compromised location: 'TOS Sampling Location Compromised: TCK – [Root Cause Description]'
    a. Example: 'TOS Sampling Location Compromised: TCK – Could not access plot for 6 consecutive bouts due to persistent flooding'

If field staff suspect that the criteria for a compromised sampling location has either been met or is likely to be met based on conditions on the ground, they should submit an incident in ServiceNow.

## SOP D Data Entry and Verification

Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific applications as they are being collected, whenever possible, to minimize data transcription errors and improve data quality. Mobile devices should be synced at the end of each field day, where possible. Alternatively, devices should be synced immediately upon return to the Domain Support Facility.

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. Data collected on paper data sheets must be transcribed within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

Be sure to enter data for all plots within a bout even if collected on a different schedule than originally planned. If an entire bout is missed, one sampling impractical record for each plot missed should be created, and a problem ticket should be issued.

*Quality Assurance*

Data Quality Assurance (QA) is an important part of data collection and ensures that all data regarding observations and samples are accurate and complete. This protocol requires that certain QA checks be conducted in the field (i.e., before a field team leaves a plot or site), while others can be conducted at a later date in the office (typically within a week of collection). Field QA procedures are designed to prevent the occurrence of invalid data values that cannot be corrected at a later time, and to ensure that data and/or sample sets are complete before a sampling window closes. Incomplete data and/or sample sets cannot be supplemented by subsequent sampling efforts if the sampling window has closed. Invalid meta-data (e.g., collection dates, plotIDs) are difficult to correct when field crews are no longer at a sampling location.

Office QA procedures are meant to ensure that sampling activities are ***consistent*** across bouts, that sampling has been carried out to ***completion***, and that activities are occurring in a ***timely*** manner. The Office QA will also assess duplicative data to maintain data ***validity*** and ***integrity***.

- A template QAQC checklist for the TCK protocol is available on Sharepoint and can be modified to accommodate site-specific needs.
- All QA measures needed for this protocol are described in the Data Management Protocol (RD[08]).

**Before samples ship to external facilities and/or their digital records load to the NEON database, the data must undergo thorough quality checks**. The steps needed to accomplish this are outlined in the TCK QC Checklist, which is available on the NEON SSL.

**Data and sample IDs must be entered digitally and quality checked prior to shipping samples to an external lab.**

## SOP E Sample Shipment

Information included in this SOP conveys science-based packaging, shipping, and handling requirements, not lab-specific or logistical demands. For that information, reference the NEON Protocol and Procedure: Shipping Ecological Samples and Equipment protocol (RD[10]) and the Domain Chemical Hygiene Plan and Biosafety Manual (AD[03]).

1. Follow sample shipping timelines in Section 4 to maintain appropriate sample hold times and storage conditions.
    - Discrepancies between this protocol document and the Shipping Protocol should be communicated to Science.
2. Follow instructions in the NEON Protocol and Procedure: Shipping Ecological Samples, Sensors, and Equipment in order to ship samples to external laboratories or the biorepository (RD[10]).

### E.1 Supplies/Containers

Double-bag the tubes containing samples using minimum 2-mil watertight plastic bags with absorbent liner inserted into the outer bag, and close securely. If shipping via the DeMinimus Exception for Non-Infectious Specimens, the outer bag must be heat sealed. Heat sealing is optional for other shipment methods. Fill all void space with bubble wrap to absorb any spills and prevent movement. If parafilm is used, ensure that it is used correctly with the film going over the outside of the cap and vial (e.g., NOT underneath on the threads). Putting parafilm on the threads can lead to gaps that allow ethanol and larval ticks to leak into the bag.

### E.2 Conditions

Following post-processing, samples should be stored at 25°C in vials containing 95% ethanol until they are shipped to an external facility. Samples should be shipped via ground shipping methods.

### E.3 Grouping/Splitting Samples

All samples collected during each bout must be shipped together. Sample vials containing samples collected as part of the same bout can be taped or rubber-banded together, or placed in a separate bag, to allow them to be easily inventoried and sorted at the external facility.

### E.4 Return of Materials or Containers

Be sure to include instructions to external facilities on how to return reusable materials. CLA can provide details.

### E.5 Shipping Inventory

Each shipment must be accompanied by a hard-copy shipping manifest AND a corresponding electronic version of the manifest. Place the hard copy shipping manifest in a resealable plastic bag on top of Styrofoam, and send the electronic copy to the CLA contact **and** the receiving laboratory using the Stork Shipment Verification Tool. The electronic manifest should be emailed to the taxonomic ID facility as soon as possible after a batch of samples has been shipped.

- Follow instructions in the NEON Protocol and Procedure: Shipping Ecological Samples and Equipment to ship samples to external laboratories or the biorepository (RD[10]).

## 8 REFERENCES

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Eisen, R.J., L. Eisen, N.H. Ogden, and C.B. Beard. 2016. Linkages of weather and climate with *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae), enzootic transmission of *Borrelia burgdorferi*, and Lyme Disease in North America. J Med Entomol. 53(2):250-61. doi: 10.1093/jme/tjv199

Ogden, N.H., G. Pang, H.S. Ginsber, G.J. Hickling, L. Beati, and J.I. Tsao. 2018. Evidence for geographic variation in life-cycle processes affecting phenology of the Lyme disease vector *Ixodes scapularis* (Acari: Ixodidae) in the United States. J Med Entomol. doi: 10.1093/jme/tjy104

# APPENDIX A QUICK REFERENCES

## A.1 Collecting Tick Specimens

WALK SLOWLY!

**STEP 1** – Check yourself for ticks, remove with duct tape, and discard.

**STEP 2** – Start sampling at one corner of the plot.

**STEP 3** – Drag cloth SLOWLY for 5-10 meters.

**STEP 4** – Stop and inspect drag cloth. Collect ticks into vial containing 95% ethanol. Keep larvae separate from other tick life stages.

**STEP 5** – Verify that vial has the correct sampleID and scan the barcode into the mobile application.

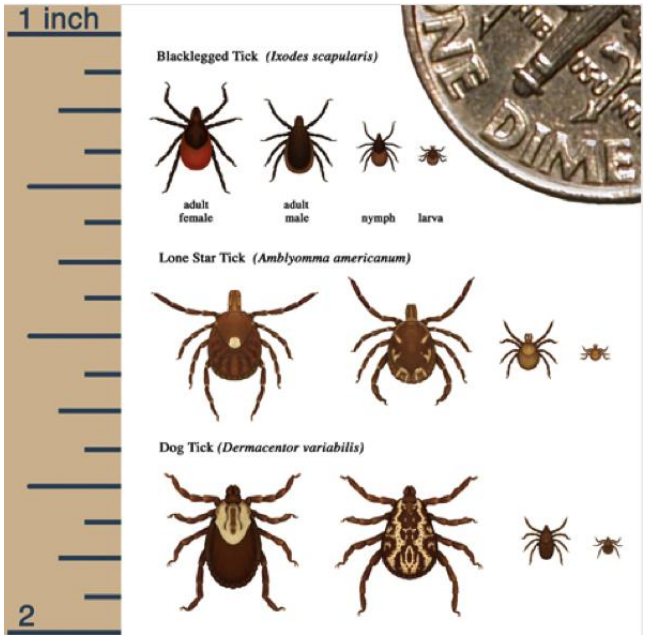
**STEP 6** – Collect larval ticks with weak painter's tape if necessary. Store in 95% ethanol for transport back to lab (ticks collected on tape only).

**STEP 7** – Repeat drag and collection cycle until you have sampled the entire perimeter of the plot (i.e. returned to the plot corner where you began your sampling).

**STEP 8** – Store specimen vials in cooler with ice packs.

**STEP 9** – After leaving the plot, check yourself for ticks, remove with duct tape, and discard.

**Tick Life Stages**



# APPENDIX B REMINDERS

## B.1 Getting Ready for Sampling

**Equipment: Be sure to…**

- ☐ Inspect drag cloth for tears and ticks.
- ☐ Check that binder clips are attached to dowel.
- ☐ Print Tick Sampling Datasheet.
- ☐ Bring a synced and charged tablet that has the Tick application loaded.
- ☐ Upload sample coordinates to GPS and obtain maps.
- ☐ Bring all supplies and extras.
- ☐ Check your pace. Can you accurately pace 5-10 meters?

**Personal Safety: Protect yourself by…**

- ☐ Wearing appropriate clothing.
- ☐ Tucking pant legs into socks.
- ☐ Using tape to seal gaps.
- ☐ Applying insect repellent: at least 30 min before going into field; away from sampling equipment.

**You are collecting live ticks.**
**If you choose to use insect repellent, apply it at least 30 minutes PRIOR to heading to the field site. Wash hands thoroughly with soap and water after applying insect repellent to avoid transferring repellent to sampling equipment.**

## B.2 Collecting Quality Tick Data

**Dragging: Remember to…**

- ☐ Check yourself for ticks BEFORE you start dragging.
- ☐ Sample only under dry conditions.
- ☐ Keep drag cloth relatively flat on ground.
- ☐ SLOW DOWN! Your pace is probably too fast.
- ☐ Remain on a path that traces the shortest straight-line distance between plot corners.
- ☐ Associate the barcode identifier with the electronic record.
- ☐ Include ticks found on your clothes while sampling in your specimen vial.
- ☐ Store tick samples in cooler with ice packs.

**Before leaving drag site, check that…**

- ☐ Field portion of datasheet or electronic record is complete.
- ☐ All ticks have been removed from drag cloth and your person(s).
- ☐ Drag cloth is stowed in plastic bag for transport to next site.

**At the end of the day, limit your exposure to ticks by…**

- ☐ Putting your field clothes in a dryer to kill ticks, or if not possible, stowing them in a plastic bag to contain ticks.
- ☐ Checking yourself for ticks.

## APPENDIX C ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The dates in **Table 7** below are estimated from satellite MODIS-EVI phenology data averaged from 2012-2021 (Didan 2023), with the exception that dates for D04 and D20, which are relatively invariant with respect to greenness, are derived from precipitation data. The season is bounded by increasing green-up as the start date and the mid-point between decreasing greenness and minimum greenness as the end date. If sites experience two peak greens, the start date is based on the first cycle of greening and the end date is based on the second cycle. Estimates for the start and stop dates of sampling are provided for each site. For convenience, this table also provides: the expected number of bouts based on the estimated seasonal duration and presence of ticks. These dates are estimates and local conditions may vary. If the listed start date passes and no observable green-up has occurred, then the start of the sampling season may be delayed until green-up is observed such that the estimated number of bouts occur. Completion of the sampling season should also coincide with the observation of dormancy in the field; if dormancy occurs prior to the end date listed in the table below, the sampling season may be terminated before the estimated end date. The expected number of bouts at the intensity level most common at a given site are included below for reference (both are included if the site could reasonably be expected to shift between high or low intensity). However, scheduling should always follow the start and end dates in this table. ***Bouts should never be canceled to achieve the expected number of bouts in the table***.

However, if green-up or dormancy at a site differs by more than one month from the listed estimated dates below (either earlier or later), issue a problem ticket to NEON Science.

Note: MODIS data are of limited utility for tropical sites (e.g., D04). For these locations, a six-month window of sampling has been selected based on patterns of precipitation at the site.

**Table 7**. Estimated sampling dates based on historical 'green-up' dates.

| Domain | Site | Start | End | Expected Number of Bouts Low Intensity | Expected Number of Bouts High Intensity |
|---|---|---|---|---|---|
| 1 | BART | 21-Apr | 24-Sep | 3 | 7 |
| 1 | HARV | 21-Apr | 25-Sep | | 7 |
| 2 | BLAN | 23-Mar | 3-Oct | | 9 |
| 2 | SCBI | 2-Apr | 24-Sep | | 8 |
| 2 | SERC | 27-Mar | 1-Oct | | 8 |
| 3 | DSNY | 7-Mar | 23-Sep | 4 | 9 |
| 3 | JERC | 5-Apr | 29-Sep | 4 | 8 |
| 3 | OSBS | 12-Mar | 16-Sep | | 8 |
| 4 | GUAN* | 1-Mar | 13-Oct | 5 | |
| 4 | LAJA* | 1-Mar | 13-Oct | 5 | |
| 5 | STEI | 26-Apr | 19-Sep | | 6 |

| Domain | Site | Start | End | Expected Number of Bouts Low Intensity | Expected Number of Bouts High Intensity |
|---|---|---|---|---|---|
| 5 | TREE | 26-Apr | 19-Sep | | 6 |
| 5 | UNDE | 29-Apr | 17-Sep | 3 | 6 |
| 6 | KONA | 5-Apr | 28-Sep | 4 | 8 |
| 6 | KONZ | 14-Apr | 24-Sep | | 7 |
| 6 | UKFS | 28-Mar | 26-Sep | | 8 |
| 7 | GRSM | 3-Apr | 25-Sep | 4 | 8 |
| 7 | MLBS | 15-Apr | 24-Sep | | 7 |
| 7 | ORNL | 24-Mar | 17-Sep | | 8 |
| 8 | LENO | 18-Mar | 20-Sep | 4 | 8 |
| 8 | DELA | 14-Mar | 16-Sep | | 8 |
| 8 | TALL | 24-Mar | 18-Sep | | 8 |
| 9 | DCFS | 4-May | 27-Sep | | 6 |
| 9 | NOGP | 20-Apr | 28-Sep | 3 | 7 |
| 9 | WOOD | 12-May | 28-Sep | | 6 |
| 10 | CPER | 6-Apr | 28 Aug | 3 | |
| 10 | RMNP | 1-May | 14-Sep | 3 | 6 |
| 10 | STER | 27-Mar | 3-Sep | 3 | |
| 11 | CLBJ | 13-Mar | 28-Sep | | 9 |
| 11 | OAES | 8-Mar | 29-Nov | | 12 |
| 12 | YELL | 14-Apr | 17-Sep | 3 | 7 |
| 13 | MOAB | 15-May | 29-Sep | 3 | |
| 13 | NIWO | 4-May | 9-Sep | 3 | |
| 14 | JORN | 22-Mar | 12-Oct | 4 | 9 |
| 14 | SRER | 1-Apr | 16-Oct | 4 | 9 |
| 15 | ONAQ | 31-Mar | 1-Aug | 2 | 5 |
| 16 | ABBY | 28-Mar | 16-Sep | | 8 |
| 16 | WREF | 21-Mar | 18-Sep | | 8 |
| 17 | SJER | 5-Oct | 11-May | 6 | |
| 17 | SOAP | 26-Mar | 3-Sep | 3 | 7 |
| 17 | TEAK | 17-Apr | 19-Sep | 3 | |
| 18 | BARR | 21-May | 9-Sep | 2 | |
| 18 | TOOL | 7-May | 27-Aug | 2 | |
| 19 | BONA | 3-May | 1-Sep | 2 | |

| Domain | Site | Start | End | Expected Number of Bouts Low Intensity | Expected Number of Bouts High Intensity |
|---|---|---|---|---|---|
| 19 | HEAL | 25-Apr | 5-Sep | 3 | |
| 19 | DEJU | 1-May | 3-Sep | 2 | 5 |

* sites where precipitation data were used in lieu of MODIS data

## APPENDIX D SITE-SPECIFIC INFORMATION

The sections below provide site-specific, alternative plot establishment or sampling guidelines.

### D.1 DELA

Sampling discontinued after the 2025 season due to planned site decommissioning.

### D.2 GUAN – Guidelines for Alternative Tick Sampling Plot Establishment

Field Operations staff will identify a target six plots (transect paths) within the TOS sampling boundary where tick sampling is feasible, in accordance with the following guidelines:

1. Required: A minimum area of 80 $m^2$ (with an ideal target of 160 $m^2$) can be sampled using either the flagging or dragging method. If necessary, the area may consist of multiple segments, each preferably within 10 meters of another segment.
2. Strongly preferred: The majority (50% or more) of the area is located within:
    - 300 m from the center of a mammal plot,
    - 300 m from the center of a bird plot, OR
    - 300 m from the center of a Distributed Base Plot

Ideally each targeted sampling area will be adjacent to all three plot types, but the order reflects priority (mammals are the first preference). Ideally both this criterion AND the Distance from other TOS plots criterion (**Table 8**) will be met, but if this is not possible, this criterion takes higher priority.

3. Preferred: Sampling does not cross roads or frequently-used paths to other plots. If no other options are available, plots may be located linearly along the edge of a dirt road or path, where vegetation or leaf litter can be sampled.

For each sampling area identified, Field Operations staff will provide to Science staff:

- A line (vector file) of the sampling path (preferred), OR
- Start, end, and change-of-direction points from which the sampling path can be approximated with linear segments.

Other than these changes in plot establishment, the tick sampling process remains the same. This includes the fixed nature of these locations until sampling is no longer possible (issue a problem ticket) or instructed otherwise.

**Table 8**. Standard vs. Alternative Tick Plot Location Criteria.

| Parameter | Standard | Alternative |
|---|---|---|
| **Plot Size** | 160m transects around a 40m by 40m plot | 160m transects of any shape |
| **Maximum distance from roads** | None | No change |
| **Minimum distance from Paved Roads** | Avoid high traffic areas | No change |
| **Minimum distance from Dirt Roads** | Avoid high traffic areas | Adherence to standard preferred, but not required |
| **Distance from Buildings** | Avoid high traffic areas | No change |
| **Distance from oil pads** | NA | No change |
| **Distance from other TOS plots** | Edge of plot is 150m from tower, phenology, and mammal plots. Centroid of the plots is 150m from a base plot. Can be within a bird grid, not on bird points. 50 m from a mosquito point. | Adherence to standard preferred, but not required |
| **Distance from same type of plot** | 500m, centroid to centroid | No change |
| **Vegetation characteristics** | 40m by 40m plot matches NLCD definition | Minimum-area polygon matches NLCD definition |
| **Stratification** | Proportional to NLCD dominant vegetation types | No change |
| **Collocation needs** | Collocated with target distributed base plots | Collocated with mammal, bird, or distributed base plots |
| **Placement Method** | Follow the M_Order list of accepted base plots. A random azimuth is determined at a distance of 150m from the base plot centroid. This distance can be shifted +/- 15m. If the first azimuth does not work 2 more random directions are tried before moving onto the next M_Order. If the target number of tick plots is still not met after going through all target base plots then grids can be placed subjectively 150m +/- 15m from the target base plot, starting at the beginning of the M_Order list again. | Locate subjectively where sampling is possible, preferably within 300 m from the center of a mammal, bird, or distributed base plot. |
| **Target number of plots** | 6 | No change |
| **Contingency # of plots** | 0-100% | No change |
| **Streams** | A stream cannot bisect the transect | Avoid placing plot segments more than 10 m apart |
| **Post Processing Accuracy (m)** | 2 | No change |
| **Comments** | Effort is made to avoid having tick plots be in between the road and another TOS plot | Avoid having tick plots intersect frequently-used paths |

## APPENDIX E EQUIPMENT

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

**Table 9**. Equipment list – Preparation for field sampling.

| Supplier/ Item No. | Exact Brand | Description | Purpose | Quan-tity |
|---|---|---|---|---|
| | N | Ice pack, 0°C | Pre-freeze for field preservation of samples | Variable |
| | N | All weather copy paper | Print datasheets, prepare internal vial labels (pre-print or use pencil) | 5 |
| | N | Waterproof adhesive label or label tape | Prepare external vial labels (pre-print or use permanent marker) | Variable |
| | N | Ethanol, 190 proof (95%); 55-gallon drum | Weaken packing tape adhesive, to remove larval ticks | Variable |
| | N | Ethanol, 190 proof (95%); 5-gallon carboy | As above. RD[05] restricted to reduced amounts of ethanol on hand. | Variable |

**Table 10**. Equipment list – Field sampling a single bout.

| Supplier/ Item No. | Exact Brand | Description | Purpose | Quan-tity |
|---|---|---|---|---|
| | N | Cooler, 16 qt | Chill perishable samples in field | 1 |
| | N | Forceps (with flagging or lanyard) | Collect ticks | 2 |
| | N | Clipboard | Hold and write on datasheets | 1 |
| | N | Pencils | Write on datasheets and internal labels | 3 |
| | N | GPS receiver, recreational accuracy, e.g. Garmin Etrex20x | Navigate to sampling location | 1 |
| | N | Ice pack, 0°C | Chill perishable samples in field | 3 |
| | N | Magnifier hand lens, 2X/5X | Aid in tick identification | 1 |
| | N | Measuring tape, minimum 50 m | Measure deviations from the drag path | 1 |
| | N | Sinker weights for tick drag cloth assembly | Weigh drag cloth to maintain contact with ground | 5 |

| Supplier/ Item No. | Exact Brand | Description | Purpose | Quan-tity |
|---|---|---|---|---|
| Contact Procurement; EB03180000 | Y | Tick drag cloth assembly | Collect ticks | 2 |
| | N | Alcohol-free hand wipes | Remove repellent residue | 2 |
| | N | Duct tape | Remove and discard ticks not being archived. Note duct tape should never be used to collect ticks destined for archive | 1 |
| | N | All-weather copy paper | Internal labels for sample vials (pre-print or use pencil) | 1 |
| | N | Waterproof adhesive label or label tape | External labels for sample vials (pre-print or use ethanol-proof permanent marker) | Variable |
| | N | Scissors | Cut labels | 1 |
| | N | Weak painter's tape | Collect larval ticks from cloth | 1 roll |
| | N | Mosquito repellent, up to 40% DEET | Protect personnel from insect bites | 1 |
| | N | Permanent marker, archival & ethanol-safe | Write external labels for sample vials | 1 |
| | N | Resealable plastic bag, 1 gal, 4 mil | Organize sample tubes | 6 |
| | N | Rubber band | Organize sample tubes | Variable |
| | N | Survey marking flag, PVC or fiberglass stake | Delineate sampling area | 4 |
| | N | Resealable plastic bag, 1 gal, 4 mil | Store and transport drag cloths | Variable |
| | N | Tubes with caps, 50 mL, or larger as needed | Prepare pre-filled tubes with 95% ethanol for soaking tape with larval ticks. Do not send 50mL vials to taxonomy laboratory | Variable |
| | N | Tubes, Microcentrifuge, 1.5-2 mL, 10 mL flat bottom | Prepare pre-filled sample vials with 95% ethanol solution. Only this size and type of vial should be sent to the taxonomy laboratory | 6 |
| | N | Tablet (mobile data entry) with Tick sampling application downloaded | Record data | 1/plot |

| Supplier/ Item No. | Exact Brand | Description | Purpose | Quan-tity |
|---|---|---|---|---|
| | N | Spare Tablet battery | | 1 |
| RD[06] | N | Field datasheet | Record data | 1 |

**Table 11**. Equipment list – Laboratory processing and analyses.

| Supplier/ Item No. | Exact Brand | Description | Purpose | Quan-tity |
|---|---|---|---|---|
| | N | Artist paintbrush | Transfer ticks to tubes | 2 |
| | N | Forceps | Transfer ticks to tubes | 2 |
| | N | Copy paper, white | Aid in visibility of ticks | 1 |
| | N | Waterproof adhesive label or label tape | External vial labels (pre-print or use permanent marker) | Variable |
| Grainger; 4TKE5, 5GUU1 or similar | N | Liquid laundry detergent, fragrance free | Wash tick drag cloth | 1 |
| | N | Resealable plastic bag, 1 gal, 4 mil | Organize sample tubes | Variable |
| | N | Rubber band | Organize sample tubes | Variable |
| | N | Tubes, 1.5-2 mL with screw-top cap and O ring, flat bottom | Store ticks in 95% ethanol solution for shipping | Variable |
| | Y | Adhesive barcode labels (Type I) | Labeling sample containers with barcode-readable labels [Note: container curvature will not permit these labels to be used on vials smaller than 10mL] | 1 sheet |
| | N | Synced data entry tablet | Record data | |
| RD[06] | N | Completed field datasheet | Record data | 1 |